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Effects of Ozone on the Regrowth and Energy Reserves of a Ladino Clover-Tall Fescue Pasture

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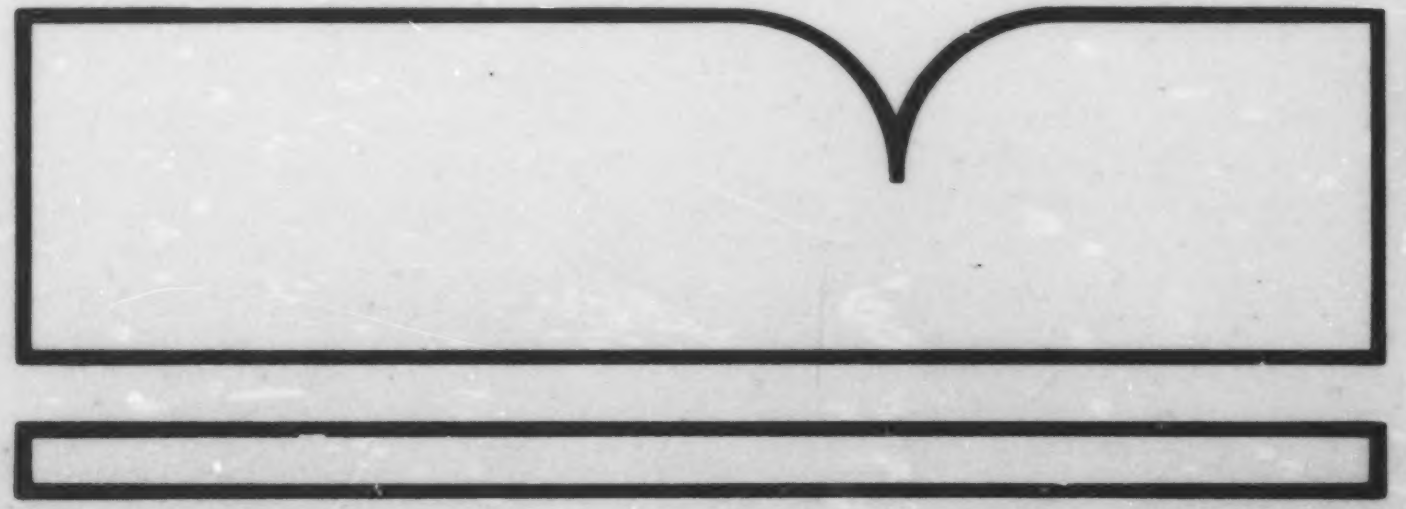

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16. ABSTRACT

A ladino clover and tall fescue pasture was established in September 1983 to determine the impact of ozone and available soil moisture on plants grown in open-top field chambers and exposed for 12 h daily to O_3 from April through October in 1984 and 1985. Samples were removed periodically to measure above- and below-ground biomass and energy reserves of the clover and fescue. At the final harvest, clover was the dominant species in below-ambient O_3 while fescue was the dominant species at ambient and above-ambient O_3 concentrations.

Ladino clover shoot and root biomass was reduced by O_3 for all harvest when compared to clover grown in the charcoal-filtered-air. Statistically-significant O_3 effects were observed on clover shoots prior to roots.

For most harvests, the energy reserves of ladino clover roots were suppressed by increasing O_3 concentrations. Clover shoot starch levels were not greatly affected by O_3 or moisture. Energy reserves of tall fescue were not affected by O_3 or moisture. Total soluble sugars in both species were generally unaffected by O_3 or moisture.

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EFFECTS OF OZONE ON THE REGROWTH AND ENERGY RESERVES OF A LADINO CLOVER–TALL FESCUE PASTURE

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SUMMARY

(1) A ladino clover (*Trifolium repens* L. c.v. Regal) and tall fescue (*Festuca arundinacea* Schreb. c.v. Kentucky 31) pasture was established in September 1983 to determine the impact of ozone and available soil moisture on plants grown in open-top field chambers and exposed for 12 h daily to O_3 (ranging from 0·57 to 2·00 times the average seasonal concentration in ambient air) from April through October in 1984 and 1985.

(2) Samples were removed periodically to measure above- and below-ground biomass and energy reserves of the clover and fescue. At the final harvest (22 October 1985), clover was the dominant species in below-ambient O_3 (83% of total biomass; live roots + live shoots) while fescue was the dominant species at ambient and above-ambient O_3 concentrations (95% of the total biomass).

(3) Ladino clover shoot and root biomass was reduced by O_3 for all harvests when compared to clover grown in the charcoal-filtered-air (0·30 $\mu l\ l^{-1}$ O_3). Statistically-significant O_3 effects were observed on clover shoots prior to roots. Significant soil moisture effects were observed on clover shoot biomass only in July 1985. Significant moisture effects on clover root biomass were not observed until July and August of 1985. Fescue shoot and root biomass production increased with increasing O_3 levels for all but the final harvest. Significant moisture effects on fescue shoot and root biomass were observed at various times in 1985.

(4) For most harvests, the energy reserves (starches) of ladino clover roots were suppressed by increasing O_3 concentrations. Clover shoot starch levels were not greatly affected by O_3 or moisture. Energy reserves of tall fescue (starch & fructosan) were not affected by O_3 or moisture. Total soluble sugars (glucose + fructose + sucrose) in both species were generally unaffected by O_3 or moisture.

INTRODUCTION

Mixed stands of legumes and grasses provide a high-quality forage for livestock. In most temperate regions, white clover (*Trifolium repens* L.) is the major pasture legume (Carlson, Gibson & Baltensperger 1985). In the south-eastern United States, ladino clover and tall fescue (*Festuca arundinacea* Schreb.) are commonly grown together. However, this is an unstable mixture because ladino clover declines within a few years after planting (Blake, Chamblee & Woodhouse 1966; Gibson & Hollowell 1966; Dobson, Fisher & Beaty 1976). A number of biotic and abiotic factors including fungal, viral and nematological diseases, insect herbivory, poor management practices (primarily inappropriate harvesting and fertilization procedures), plant competition, allelopathy, poor drought tolerance, and intolerance to ozone (O_3) have been suggested as a cause for this loss of clover (Sprague & Sullivan 1950; Garren 1954, 1955; Westbrooks & Tesar 1955;

Lucas & Harper 1972; Leffel & Gibson 1973; Bennett & Runeckles 1977; Haynes 1980; Kochhar, Blum & Reinert 1980; Carlson, Gibson & Baltensperger 1985).

Decline of ladino clover populations at ambient (0·05 $\mu l\ l^{-1}$ 7 h mean) or slightly elevated O_3 levels has been observed under field conditions (Blum *et al.* 1983). Ozone has been shown to reduce shoot and root growth, nitrogen fixation, concentrations of total non-structural carbohydrate, % estimated digestibility and mineral content of ladino clover in a number of field, greenhouse, and controlled-environment studies (Letchworth & Blum 1977; Kochhar, Blum & Reinert 1980; Blum, Smith & Fites 1982; Montes, Blum & Heagle 1982; Blum, Mrozek & Johnson 1983; Blum *et al.* 1983; Montes *et al.* 1983). Tall fescue, however, has been found to be more tolerant to O_3 and drought than ladino clover in these and other studies (Hagan *et al.* 1957; Haynes 1980; Johnson & Raguse 1985).

A number of studies have demonstrated reductions in net photosynthesis and alterations in respiration and carbohydrate pools (storage carbohydrates and soluble sugars) in a variety of species following exposure to O_3 (Tomlinson & Rich 1967; Hill & Littlefield 1969; Tingey, Fites & Wickliff 1973; Blum, Smith & Fites 1982; Blum, Mrozek & Johnson 1983; Koziol 1984). Ozone-induced suppression of root biomass probably results from a reduction of assimilate translocation from leaves to roots. Blum, Mrozek & Johnson (1983) reported reduced allocation of ^{14}C from leaves to roots of ladino clover following O_3 exposures (0·15 $\mu l\ l^{-1}$ for 4 h per day for 6 days, 32 days after planting) and suppressed root biomass accumulation. Blum, Smith & Fites (1982) also observed O_3 suppressed root growth and reduced total non-structural carbohydrate pools in roots and shoots of clover. Blum *et al.* (1983) suggested that O_3 reduces the survival of ladino clover during periods of stress and/or overwintering by reducing essential energy reserves. To date, no field study has been conducted to assess the impacts of O_3 on both above- and below-ground biomass and energy reserves of a mixed pasture.

The primary objective of this study was to determine the effects of O_3 on the regrowth and energy reserves (soluble sugars, starch, and fructosan) of the roots and shoots of ladino clover and tall fescue. A secondary objective was to determine how levels of soil moisture might alter the response of clover and fescue to O_3. O_3 primarily enters the plant via stomata, and water-stressed plants may thus be less affected by O_3.

MATERIALS AND METHODS

Plant field culture

Ladino clover (cv. Regal) and tall fescue (cv. Kentucky 31) seeds were sown in a field (sandy clay loam, Appling-Cecil Association) 6 km south of Raleigh, North Carolina, on 15 September 1983 at rates of 5 and 13 kg ha^{-1}, respectively. Clover seeds were inoculated with *Rhizobium trifolii* Dangeard at planting. The field was fertilized at seeding according to soil test recommendations. Seedlings of both species emerged 1 week after planting. The pasture was mowed to a height of 7·5 cm on 9 January 1984.

In March of 1984, the field was divided into four areas based on topography and depth to the B-horizon. Soil moisture release curves for A and B horizon material were generated for each area from a composite sample of eight to ten cores (each 2·54 cm diameter × 30 cm deep) taken approximately 0·3 m apart in each area. These curves were then used to convert water content of the soil to the moisture tension values, which were used to determine when to irrigate the plots (see later section).

On 2 April 1984 the pasture was divided into two sections based on the uniformity of clover (since it was the dominant species in the plant canopy). Fourteen 3-m diameter

12 weeks, before a significant reduction in TNC occurred. Exposure to lower O_3 concentrations did not significantly reduce the % TNC of tall fescue.

In summary, clover decline was accelerated by O_3. This was expressed in decreased production of roots and shoots of ladino clover. The storage carbohydrates (starch) levels of this O_3-sensitive species were reduced, while soluble sugars levels were unaffected by O_3 exposures. Tall fescue was not directly affected by O_3 exposures either in its energy reserves (storage carbohydrates and soluble sugars) or biomass production but was indirectly stimulated under high O_3 treatments.

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plots were chosen within each section based on similarity in clover distribution. Although smaller in height and with lower above-ground biomass per unit area than clover, fescue appeared to be evenly distributed throughout the pasture. Open-top field chambers (3 m diameter × 2·4 m tall) (Heagle, Body & Heck 1973) were placed over each of the twenty-eight plots on 3 April 1984. The clear polyvinyl-chloride film panels were not installed until 24 April 1984. Six contiguous quadrats (each 36 × 51 cm) were permanently marked within the centre of each plot. The average density of both species in the pasture was approximately 60–65 seedlings m^{-2} on 15 April 1984.

Details of the O_3 dispensing and monitoring system used in this study have been described by Heagle *et al.* (1979, 1986). A randomized complete block design with a factorial arrangement of seven O_3 levels and two moisture levels was used. Ozone treatments included a non-chambered ambient air treatment (AA); and chambers that received charcoal-filtered air (CF), unfiltered air (NF), or NF air to which O_3 was added to produce concentrations averaging 1·25, 1·50, 1·75, or 2·00 times that of ambient air (i.e. 1·25 × AA, 1·50 × AA, 1·75 × AA, or 2·00 × AA). Chambers provided a mean air velocity of 2·5 km h^{-1} in the plant growth area. Mean air temperature during daylight hours was 0·71 °C above ambient. Mean photosynthetic flux density (400–700 nm) in chambers was 88% of ambient. Artificially-generated O_3 (produced by 'silent arc' electric discharge in oxygen) was added for 12 h per day during 08.00–20.00 h whenever the ambient O_3 concentration exceeded 0·03 μl^{-1} during 26 April to 18 October 1984 and 13 April to 22 October 1985. O_3 additions were not made during plot harvests, irrigations, or pesticide applications.

Soil moisture (0–30 cm layer) was measured gravimetrically at least twice weekly and approximately 24 h after each rainfall and converted to moisture tension values using moisture release curves determined with a pressure-plate apparatus (Haines 1930). Due to severe space restrictions within the chambers (permanent quadrats and destructive root core samplings), only the 1·25 × AA and 1·75 × AA plots (a total of four plots) were monitored for soil moisture status routinely throughout the 2-year study. These two O_3 treatments were not sampled for root and shoot biomass and carbohydrate status. Two cores (2·5 cm × 30 cm) were collected from each plot. A total of four plots were sampled at each soil moisture determination. Soil removed for gravimetric moisture determinations was replaced to decrease the effects of core sampling on the rate of soil drying. All plots (AA, CF, NF, 1·50 × AA, and 2·00 × AA) were monitored on 25 June, 10 August, 7 October, and 14 October 1985. The resulting data demonstrated that soil moisture was not significantly affected by O_3 treatment. Well-watered (WW) and water-stressed (WS) plots were irrigated (2·54 cm of water from an overhead nozzle system suspended 2·4 m above the centre of each plot) when soil moisture tension values reached −0·4 MPa and −1·2 MPa, respectively. Since ambient rain could not be excluded the differences between the two soil moisture 'treatments' were greatest during periods of low rainfall.

Plots were sprayed following each harvest with Cygon 2E® (dimethoate) at 6·3 ml l^{-1} or Malathion 5EC® (malathion) at 114 ml l^{-1} to prevent insect and mite infestations. Pre- and post-season soil samples were taken each year for chemical analyses. Plots were topdressed with potassium chloride at a rate of 130 kg ha^{-1} on 12 April 1985.

Yields from permanent quadrats

Each plot was harvested when plants reached an average height of 20–25 cm (as determined by several measurements of the canopy height in all plots). This cutting

schedule was chosen to maintain an environment favourable to the persistence of clover in the pasture (Leffel & Gibson 1973; Dobson, Fisher & Beaty 1976). At each harvest, plants were clipped to 7·5 cm. Clipped shoots from the quadrats were hand-sorted into fescue, clover and weeds, oven-dried at 70 °C, and weighed.

Data from the first harvest on 18 April (Block 1) and 19 April (Block 2) (prior to the initiation of treatments) were used as a covariate in subsequent analyses. Six harvests occurred between 15 May and 22 October 1984. Differences in plant growth rates associated with only O_3 treatments were already obvious by mid-June 1984. The plants exposed to 'high' O_3 concentrations grew much slower than plants exposed to 'low' O_3 concentrations, with no observable effects on plant regrowth due to the differential watering regimes. Consequently, independent harvesting schedules were established for the AA, CF, NF, and 1·25 × AA plots versus the 1·50 × AA, 1·75 × AA and 2·00 × AA plots. The differential watering regimes did not affect plant regrowth until late 1984, with subsequent sampling dates being only slightly modified. In 1985, a pretreatment harvest was made on all plots on 9 April (Block 1) and 10 April (Block 2). Six additional harvests occurred between 13 May and 24 October of that year.

Above- and below-ground biomass production and energy reserves

The area not occupied by the permanent quadrats within each plot was used to take core samples. Edge effects were minimized by avoiding the 45-cm border along the chamber wall. All plots were sampled except for the NF + 1·25 × AA and NF + 1·75 × AA plots. Prior to each sampling, one 12-cm-diameter area was randomly chosen and marked in the east and west sections of each plot a few days after the permanent quadrat harvest (when plants were approximately 8 cm tall; times varied with low and high O_3 treatments). One day prior to the low O_3 permanent quadrat harvests, all (with the exception noted below) premarked areas (low and high O_3) were clipped at the soil level, and the shoots were separated by species and living and dead material. Living shoot material was frozen, stored on ice, and transported to the laboratory for freeze-drying. From the clipped area, a soil core 11·43 cm diameter × 20 cm deep was removed. The roots were washed free of soil and collected on sieves with 5·6, 2·0, and 1·0 mm openings. The washed roots were blotted with paper, frozen and stored at 0 °C until they could be sorted. Semi-frozen roots were sorted on trays of ice into clover, fescue, and weed species. Preliminary root sorting experiments were carried out on clover and fescue roots grown alone or in mixture, under both greenhouse and field conditions, to establish valid, reproducible visual criteria for distinguishing the roots of the two species. Separation of roots was based on colour, texture, and branching patterns. Samples were further divided into living and dead roots. Living tissue, identified by visual inspection was freeze-dried and weighed. Dead material of shoot and root samples of each species was oven-dried at 70 °C for 48 h and weighed.

Pretreatment samples (ten randomly chosen cores taken in each block outside the plots) collected on 13 April 1984 provided base-line data for shoot and root biomass, ratio of living to dead tissue for both shoots and roots, and carbohydrate levels within the tissues of each species. Since carbohydrate levels vary diurnally, plants were always sampled from 07.00 to 11.00 h (EST) throughout the study. All core samples were taken (from high and low plots) one day prior to the permanent quadrat harvest of the low O_3 plots (except on 5 June 1984 and 5 April 1985 when permanent quadrats from all O_3 treatments were harvested). A total of four cores per O_3 × moisture treatment (or eight cores per O_3 treatment) were sampled at each harvest. In 1984, in addition to the pretreatment harvest

in April, root core samples were taken within plots on 5 June, 26 July, and 26 September. In 1985, core samples were taken on 5 April, 8 July, 8 August, and 22 October.

Carbohydrate determinations

Soluble sugars

Freeze-dried root and shoot tissue of clover and fescue was ground to pass through a 40-mesh screen and stored at room temperature in air-tight glass vials within a sealed desiccator. No measurable carbohydrate degradation was detected during this storage period. Soluble sugars of clover were extracted with 80% v/v ethanol. Fescue samples were extracted with 90% v/v ethanol to remove only mono- and disaccharides, and short-chain fructans (Smith 1972, 1973). Three subsamples of freeze-dried tissue (50 mg each) were mixed with 7 ml ethanol, heated at 80 °C for 8 min, and centrifuged. Subsamples were extracted in this way three times. Supernatants from each subsample were combined for a final volume of 25 ml and stored at 0–2 °C. A 9 ml aliquot from each subsample was evaporated to dryness *in vacuo*, redissolved in 1 ml distilled water, centrifuged, and filtered (0·20 μm).

Sucrose, glucose, fructose, and short-chain fructans were quantified by high performance liquid chromatography (HPLC) equipped with a differential refractometer and a Brownlee Polypore CA column. Sterile distilled water, the solvent, flowed at a rate of 0·3 ml min^{-1}. Guard columns (C_{18} and cation/anion) maintained at 22 °C preceded the analytical column, which was maintained at 75 °C.

Starch and fructosan

The pellets remaining after the ethanol extraction (see soluble sugar determination) were resuspended in 1·5 ml deionized water, boiled for 30 min and allowed to cool to room temperature. Starch was hydrolysed to glucose by adding 1·5 ml 50 mM acetate buffer and 1·5 ml 0·2% dialysed amyloglucosidase and incubating samples at 55 °C for 24 h. The enzymatic reaction was terminated by placing sample tubes in boiling water for 5 min. Following centrifugation, glucose was determined on an aliquot of the supernatant (Nelson 1944; Clark 1964).

To determine fructosan in fescue tissue, a 1-ml aliquot of the hydrolysed starch supernatant was hydrolysed further with 0·5 ml 1·8 N H_2SO_4. Samples were boiled for 10 min, cooled in ice water for 5 min, and neutralized with 1·5 ml 0·6 N NaOH. Fructose concentration was quantified spectrophotometrically (Nelson 1944; Clark 1964). The concentration of fructosan was estimated by subtracting values of the monosaccharide produced by acid hydrolysis from those produced by amyloglucosidase hydrolysis.

RESULTS

Environmental data

Ozone concentrations, photosynthetically-active radiation (PAR), temperature (average, maximum and minimum), ambient rainfall, and irrigation for periods between samplings in 1984 and 1985 are shown in Tables 1 and 2. The major difference in environmental conditions between the two growing seasons was the amount of precipitation. Rainfall in April through October was 13 cm higher than normal in 1984, and 26 cm below normal in 1985. Soil moisture levels (MPa) for well-watered (WW) and water-stressed (WS) plots in 1984 and 1985 are presented in Fig. 1. Soil moisture tension

TABLE 1. Environmental conditions during periods preceding core harvest dates taken during 1984 in Raleigh, North Carolina

Dates*	17 May to 5 June	28 June to 26 July	9 July to 26 July	26 Aug. to 26 Sept.	7 Sept. to 26 Sept.
O_3 treatment	All	Low	High	Low	High
Ozone ($\mu l\ l^{-1}$)†					
AA	0·048 ± 0·001	0·040 ± 0·001	0·049 ± 0·001	0·047 ± 0·001	0·045 ± 0·001
CF	0·028 ± 0·001	0·022 ± 0·001		0·027 ± 0·001	
NF	0·047 ± 0·001	0·039 ± 0·001		0·045 ± 0·001	
1·50 × AA	0·079 ± 0·003		0·056 ± 0·002		0·061 ± 0·002
2·00 × AA	0·099 ± 0·004		0·071 ± 0·003		0·075 ± 0·003
PAR (μEinsteins $m^{-2}\ sec^{-1}$)	546	540	502	481	462
Air temperature (°C)					
Average	22·3	24·2	24·5	21·7	20·9
Maximum	29·1	30·0	30·1	27·6	26·1
Minimum	15·3	19·8	20·3	15·5	14·8
Rainfall (mm)	178	229	82	38	35
Irrigation‡ (mm)					
Well-watered	50	0	0	125	75
Water-stressed	50	0	0	50	25

* Represent dates preceding a harvest; does not include pretreatment harvest period.

† O_3 concentration during 08.00–20.00 h each day; AA, ambient air plots; CF, charcoal-filtered chambers; NF, non-filtered air chambers; 1·50 × AA, non-filtered air plus 1·50 times ambient O_3 concentration; 2·00 × AA, non-filtered air plus 2·00 times ambient O_3 concentration.

‡ Well-watered plots were irrigated when soil moisture reached −0·4 MPa and water-stressed plots were irrigated at −1·2 MPa.

values differed between WS and WW plots from 19 August through October 1984 and throughout most of 1985. Seasonal O_3 concentrations (12-h day means) for the 1984 growing season were 0·045, 0·025, 0·044, 0·071 or 0·088 $\mu l\ l^{-1}$ and for the 1985 season were 0·048, 0·030, 0·049, 0·075 or 0·092 $\mu l\ l^{-1}$ for AA, CF, NF, 1·50 × AA, or 2·00 × AA treatments, respectively.

Cumulative yield in permanent quadrats

The 1984 and 1985 cumulative yield of ladino clover and weed-free yield of clover + tall fescue were significantly reduced by O_3 and moisture treatments. Annual cumulative yield of clover in 1984 was significantly reduced by 11, 27, 43, 49 and 55% and in 1985 by 12, 44, 56, 73 and 75% for NF, 1·25, 1·50, 1·75 and 2·00 × AA treatments, respectively, when compared to clover yield in CF air (124 g $quadrat^{-1}$); (1984: $P < 0·0003$; 1985: $P < 0·0022$); (A. S. Heagle *et al.* unpublished). Over the 2-year period, a dramatic shift in species dominance was observed. In 1984, clover was the dominant species in ambient and below-ambient O_3 air (CF, NF and AA), while fescue was dominant in above-ambient O_3 air (1·25 × AA, 1·50 × AA, 1·75 × AA and 2·00 × AA) (Fig. 2). However, in 1985, clover was the dominant species only in CF air. The water-stress moisture treatment significantly reduced clover, weed-free (clover + fescue), and total (clover + fescue + weeds) cumulative yield in both years. Cumulative clover yield was 10 and 33% less in WS than WW plots in 1984 and 1985, respectively. Cumulative weed-free yield (clover + fescue) was 12% and 14% less in WS plots (O_3 treatments combined) than in WW plots in 1984 and 1985, respectively. Cumulative fescue yield was not significantly affected by moisture treatment in either year. Significant O_3 × moisture treatment interactions were observed only for the 1985 cumulative yield of clover.

TABLE 2. Environmental conditions for periods preceding core harvests taken during 1985 in Raleigh, North Carolina

Dates*	7 June to 8 July	18 June to 8 July	9 July to 8 Aug.	23 July to 8 Aug.	6 Sept. to 22 Oct.	25 Sept. to 22 Oct.
O_3 treatment	Low	High	Low	High	Low	High
Ozone ($\mu l\ l^{-1}$)†						
AA	0·055 ± 0·001	0·067 ± 0·001	0·049 ± 0·001	0·040 ± 0·001	0·040 ± 0·001	0·038 ± 0·001
CF	0·036 ± 0·001		0·032 ± 0·001		0·025 ± 0·001	
NF	0·056 ± 0·001		0·050 ± 0·001		0·040 ± 0·001	
1·50 × AA		0·094 ± 0·003		0·049 ± 0·001		0·051 ± 0·002
2·00 × AA		0·114 ± 0·003		0·058 ± 0·002		0·063 ± 0·003
PAR (μEinsteins $m^{-2}\ sec^{-1}$)	575	563	467	367	407	357
Air temperature (°C)						
Average	24·7	23·8	24·7	23·5	19·0	19·0
Maximum	30·2	30·2	30·8	28·9	26·9	25·8
Minimum	17·7	18·1	20·0	19·6	14·3	13·3
Rainfall (mm)	120	78	101	84	77	70
Irrigation‡ (mm)						
Well-watered	75	50	50	25	100	25
Water-stressed	50	25	25	0	50	0

* Represent dates preceding a harvest; does not include the pretreatment harvest period.

† O_3 concentration during 08.00–20.00 h each day: AA, ambient air plots; CF, charcoal-filtered chambers; NF, non-filtered air chambers; 1·50 × AA, non-filtered air plus 1·50 times ambient O_3 concentration; 2·00 × AA, non-filtered air plus 2·00 times ambient O_3 concentration.

‡ Well-watered plots were irrigated when soil moisture reached −0·4 mPa and water-stressed plots were irrigated at −1·2 mPa.

Above- and below-ground core sampling

Ozone caused a shift in species composition, as determined by total plant biomass (live roots + live shoots) (Fig. 3). The percentage of clover (core area = 102 cm^2) was highest in CF air and lowest in 2·00 × AA at the final harvests in 1984 and 1985. By the end of the study (22 October 1985), clover biomass accounted for 83, 39, 0·5, 1·8 or 0% of the total biomass in cores collected from CF, AA, NF, 1·50 and 2·00 × AA, respectively (averaged across WW and WS plots). Conversely, percentages for tall fescue were highest in 1·50 and 2·00 × AA, and lowest in CF air for both 1984 and 1985. At the final 1985 harvest, % fescue was 17, 61, 99, 98 or 100 for the CF, AA, NF, 1·50 and 2·00 × AA treatments, respectively. Suppression of fescue biomass continued throughout both growing seasons only in the CF plots. Small reductions in total clover biomass were observed in WS plots, conversely, small increases in total fescue biomass were observed in WS plots late in 1984 and 1985 seasons. These observations are consistent with the permanent quadrat data.

Seasonal patterns discussed below, for ladino clover and tall fescue are presented separately for low and high O_3 exposed plants, since the permanent quadrats and therefore the whole chamber of high O_3 exposed plants (1·50 and 2·00 × AA) were on a different harvesting schedule than the low O_3 exposed plants (AA, CF, and NF). Thus, even though all core samples were taken at the same time, regrowth of the low and high O_3 treatments were separated by time. Chamber effects (i.e. AA versus NF plots) on fescue biomass only were observed in 1985 on the following dates: 5 April (roots), 8 August (roots), and 22 October (roots & shoots) (Fig. 5). Dead root mass (both species) was generally directly proportional to living root biomass and is therefore not described.

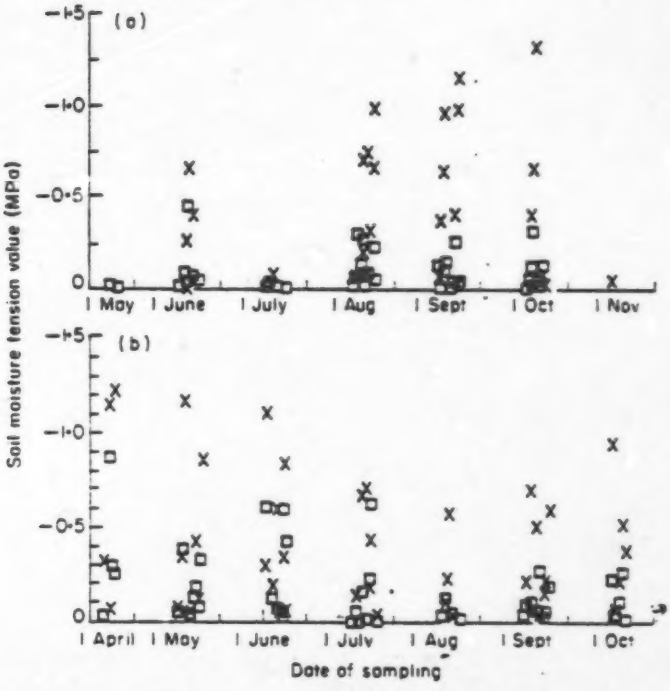

FIG. 1. Soil moisture tension values (MPa) of well-watered (□) and water-stressed (×) plots in (a) 1984 and (b) 1985. Each value represents the mean moisture status of eight samples (two soil cores from two ozone treatments, 1·25 and 1·75 times that of ambient air, and two blocks).

Ladino clover biomass

Biomass of clover shoots grown in low O_3 exhibited a bimodal pattern, i.e. a rapid regrowth in spring of both years with a subsequent decline in regrowth for the remainder of each year (Fig. 4). For all but one harvest, clover shoots from the CF plots had the highest mean dry weight per core when compared to NF and AA plots (Fig. 4). Statistically-significant O_3 effects on shoot biomass of clover grown in low O_3 air were observed only on the final 1984 and 1985 harvests. Clover grown in CF air in 1984 had 36% higher shoot weight per core than in AA and NF air on that date. In 1985, CF-air-grown clover had 66 and 99% higher shoot weight per core than AA and NF, respectively.

Plants grown in high O_3 air had a similar pattern of regrowth as plants grown in low O_3, but peak standing clover biomass occurred in high O_3 plots earlier in 1984 and later in 1985 than for low O_3 plots (Fig. 4). Clover shoot production was suppressed more by exposure to 2·00 than 1·50 × AA, for all but the first 1984 sampling (54 days after initial O_3 exposure). Statistically-significant O_3 effects on clover shoot production in 1·50 and 2·00 × AA treatments were observed on 26 July and 26 September 1984, and 5 April 1985. Shoots grown in 2·00 × AA were reduced by 36, 25, or 18%, respectively, when compared with shoots grown in 1·50 × AA. Clover was absent in cores removed from the high O_3

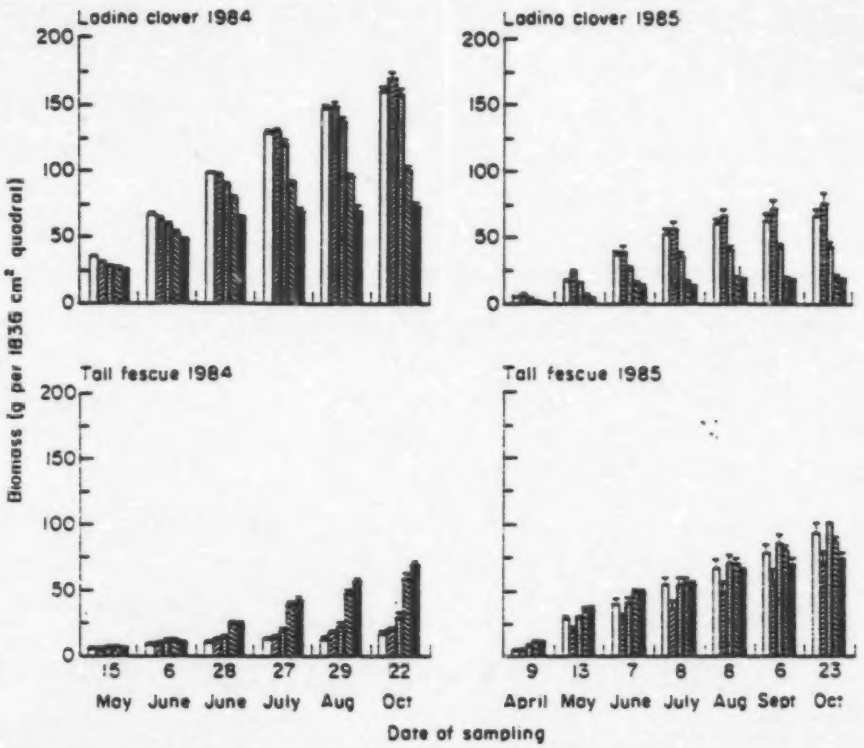

FIG. 2. The cumulative yield of ladino clover and tall fescue for each harvest in the permanent yield quadrats during 1984 and 1985. Each value represents the mean of twenty-four values, combined over moisture levels for ambient air (AA, □); charcoal-filtered air (CF, ▨); unfiltered air (NF, ▨); unfiltered air with 1·5 (■) and 2·0 (■) times the O_3 concentration of ambient air.

treatments on the final harvest in 1985. No statistically-significant moisture level effects were observed on shoot production of clover grown in either low or high O_3 air.

Root biomass of clover grown in low O_3 treatments declined slightly in 1984, increased in the spring of 1985, and subsequently declined (Fig. 3). Maximum living clover root production was observed on 5 April 1985. Root biomass of CF-grown plants was 26 and 40% higher than AA and NF-grown plants, respectively, on 26 September 1984. In 1985, O_3 effects in low O_3 grown clover were not observed until late in the season (8 August and 22 October). For the 8 August harvest, living root biomass of CF-grown plants was 57% higher than both AA and NF root biomass. For the 22 October sampling, the root biomass of CF-air-grown clover was 54 and 98% higher than the root biomass of AA and NF-grown plants, respectively. A significant moisture effect was observed on living clover root biomass of low O_3 grown plants on 8 August 1985, where WW plants produced 25% more root biomass (g core^{-1}) than WS plants. No O_3 × moisture level interactions were observed.

Root biomass of clover in the high O_3 treatments essentially declined from the start of the treatments with a small recovery during the spring of 1985 for the 1·50 × AA

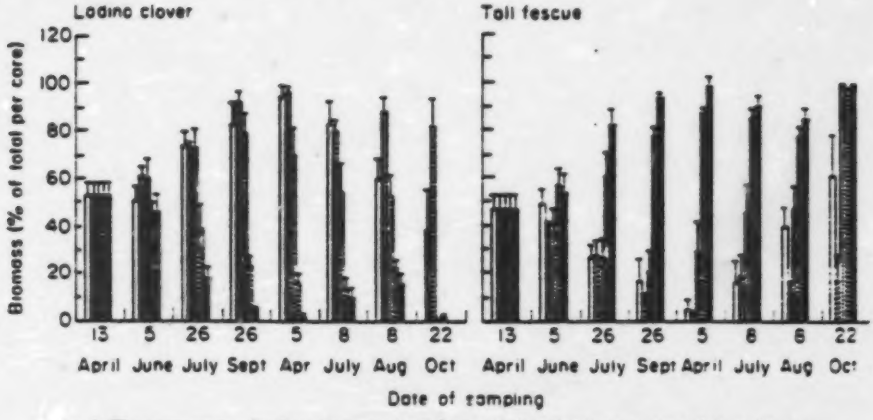

FIG. 3. The percentage of ladino clover and tall fescue in the total living root and shoot biomass from cores sampled outside permanent quadrats during 1984 and 1985. Each value represents a mean ± S.E. of eight values and is expressed as % of total dry weight per core. Each core occupied an area of 102 cm^2. Values are combined over moisture levels. Abbreviations as in Fig. 2.

treatment. By the end of the 1985 growing season, clover roots were not found in the sampling cores. For all harvests, when clover was present, living root biomass was lower for 2·00 than for the 1·50 × AA grown plants, although the decrease was not always statistically significant. Only for the final 1984 sampling (26 September) and the first 1985 sampling (5 April), were living root biomasses significantly reduced by the 2·00 × AA when compared with root biomass from the 1·50 × AA treatment. Reductions of living clover root biomass of 65% on 26 September and 84% on 5 April were observed. No significant moisture level effects on living root biomass were observed for high O_3 treated plants.

Tall fescue biomass

The shoot biomass of tall fescue, an O_3-tolerant species, was not significantly affected by the low O_3 treatments until the end of 1985. Throughout 1984, fescue shoot biomass was suppressed (by the vigorous growth of clover) when grown in CF, NF, and AA (Fig. 5). In 1985, the highest fescue shoot biomass was observed in NF plots. In CF plots, shoot biomass was suppressed (by the presence of clover) over the entire study. Significant O_3 effects on shoot production of low O_3-grown fescue were not observed until 8 August 1985. Both NF and AA-grown plants had significantly higher shoot biomass (*c.* 67%) than CF-grown plants. For the final 1985 harvest, fescue shoot biomass was 89% higher in NF than CF air ($P < 0{\cdot}01$). A significant moisture level effect on shoot biomass was observed only on 8 August 1985. WS plants had 62% more shoot biomass than WW fescue.

Shoot biomass of fescue grown in high O_3 air increased from 27 July 1984 until 5 April 1985. This was followed by a decline and subsequently by a constant regrowth. No statistically significant differences due to O_3 were observed between the shoot biomass of 1·50 and 2·00 × AA-grown plants. A significant moisture level effect was observed on 22 October 1985, with WW plants producing 34% more shoots (g $core^{-1}$) than WS plants.

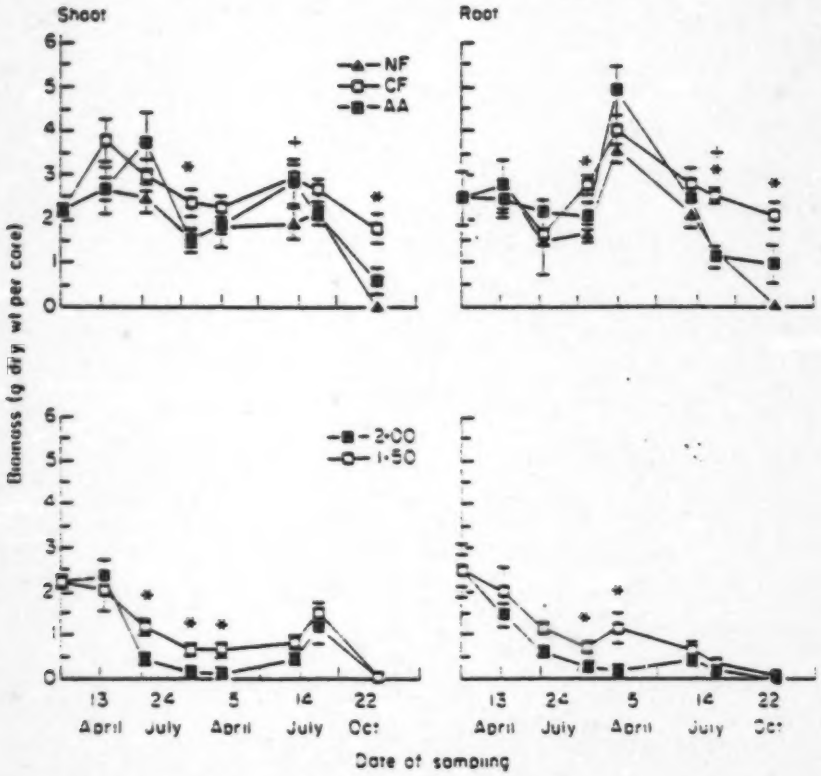

FIG. 4. Seasonal biomass production of ladino clover shoots and roots grown in either low (AA, CF, and NF) or high ozone (NF + 1·50 × AA and NF + 2·00 × AA) air. Each point represents the mean dry weight per core (g) of eight core samples ± S.E. Each core occupied an area of 102 cm^2. Statistically significant treatment effects ($P < 0·05$) are indicated by the following symbols: * for O_3 and − for moisture. Values are combined over moisture levels. Abbreviations as in Fig. 2.

Living root biomass of fescue grown in low O_3 (AA, CF and NF) air declined in 1984 (Fig. 5). However, in 1985, living root biomass increased in NF and AA plots but was essentially unchanged for CF-grown plants. For the final 1985 harvest, living root biomass of fescue grown in NF air was 92 and 53% higher than CF and AA-grown plants, respectively. Chamber effects on root biomass were observed in April, August, and October 1985. A significant moisture ($P < 0·002$) and O_3 × moisture ($P < 0·02$) effect was observed on 8 August 1985 for low O_3 grown fescue. In all the low O_3 treatments, the WS plants had higher dry weights than WW plants. Furthermore, plants treated with the combination of O_3 and WS produced the highest living biomass (i.e. NF + WS plants had more living biomass than CF + WW plants).

Living root biomass of fescue grown in high O_3 air was generally unchanged in 1984 either by time or by O_3 treatments (Fig. 5). However, living root biomass had increased dramatically by 5 April 1985. By this date, root biomass for the 1·50 and 2·00 × AA-grown plants had increased 69% and 71%, respectively, from the 26 September 1984 harvest.

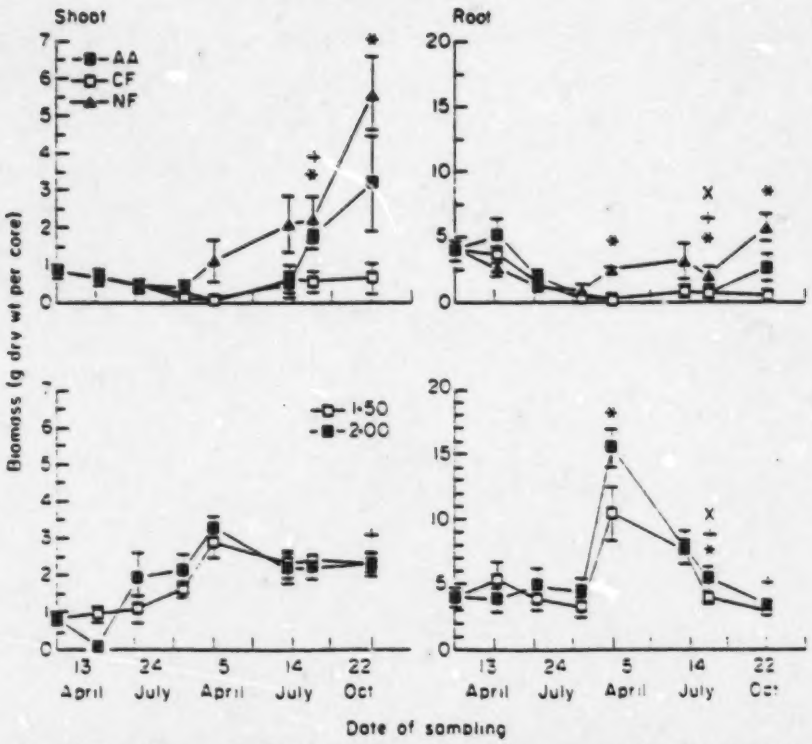

FIG. 5. Seasonal biomass production of tall fescue shoots and roots grown in either low (AA, CF, and NF) or high ozone (NF + 1·50 × AA and NF + 2·00 × AA) air. Each point represents the mean dry weight per core (g) of eight cores ± S.E. Each core occupied an area of 102 cm^2. Statistically significant treatment effects ($P < 0·05$) are indicated by the following symbols: * for O_3, – for moisture and × for O_3 × moisture effect. Values are combined over moisture levels. Abbreviations as in Fig. 2.

Following this maximum biomass peak, root biomass declined (i.e. regrowth decreased) to the final 1985 harvest. Significant O_3 effects were observed only in 1985, on 5 April and 8 August. Significant moisture ($P < 0·05$) and O_3 × moisture ($P < 0·03$) effects were observed on 8 August 1985 (i.e. 2·00 × AA + WS plants had more living roots than 1·50 × AA + WW plants).

Root–shoot ratios were calculated from biomass data obtained from core samplings and ranged from 0·54 to 2·61 for clover and from 0·37 to 7·79 for fescue. No clear seasonal pattern in root–shoot ratios of both species was observed.

Estimates of the number of cores required to detect differences of 10, 20, 30 and 50% between two means of the roots and shoots (living biomass) of clover and fescue were determined. These represented conservative estimates, with the ability to detect a Type I error set at 95% and a Type II error set at 80%. The number of cores required to determine a 50% difference ranged from 2 to 58 and 1 to 176 cores for clover and fescue, respectively. The maximum possible number of cores that could be removed from each plot was 176

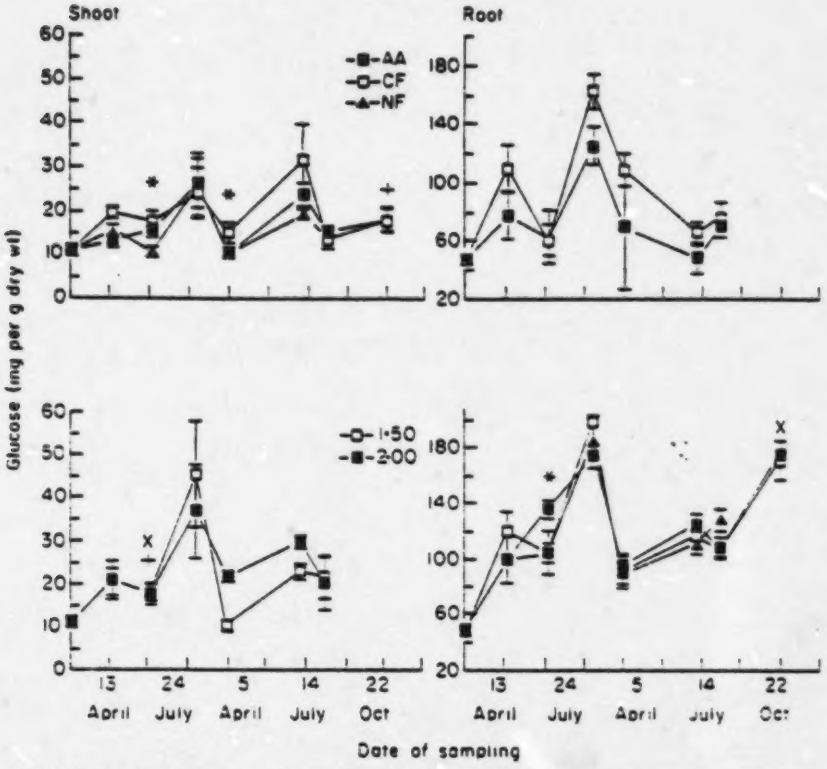

FIG. 6. Starch content (mg glucose g^{-1} dry weight) in ladino clover shoots and roots grown in either low (CF, AA, and NF) or high (1·50 × AA and 2·00 × AA) ozone air. Each point represents the mean of eight samples ± S.E. Statistically significant treatment effects ($P < 0{\cdot}05$) are indicated by the following symbols: * for O_3, + for moisture and × for O_3 × moisture effect. Values are combined over moisture levels. Abbreviations as in Fig. 2.

(excluding the area occupied by the permanent quadrats). As might be expected, the greatest number of cores were required for the least represented species in a plot.

Energy reserves

Storage carbohydrates

Ladino clover. Periods of starch accumulation and depletion were observed for both clover shoots and roots (Fig. 6). For over half of the harvests, starch was highest in clover plants grown in CF air (Fig. 6), although these increases were not always statistically significant. The starch content (mg g^{-1} dry weight) of clover shoots from CF air plots averaged over all harvests was 32% higher than the NF- and AA-grown plants (means calculated, no analysis). Significant O_3 effects on starch content of clover shoots grown in low O_3 air were observed on 26 July 1984 ($P < 0{\cdot}02$) and 5 April 1985 ($P < 0{\cdot}02$). On 26 July 1984, the starch concentration (mg g^{-1}) of clover shoots grown in CF air was 14 and 39% higher than AA- and NF-grown plants, respectively. On 5 April 1985 (both low and high O_3 were harvested at the same time), starch levels in clover shoots were 10·5 (AA, NF and

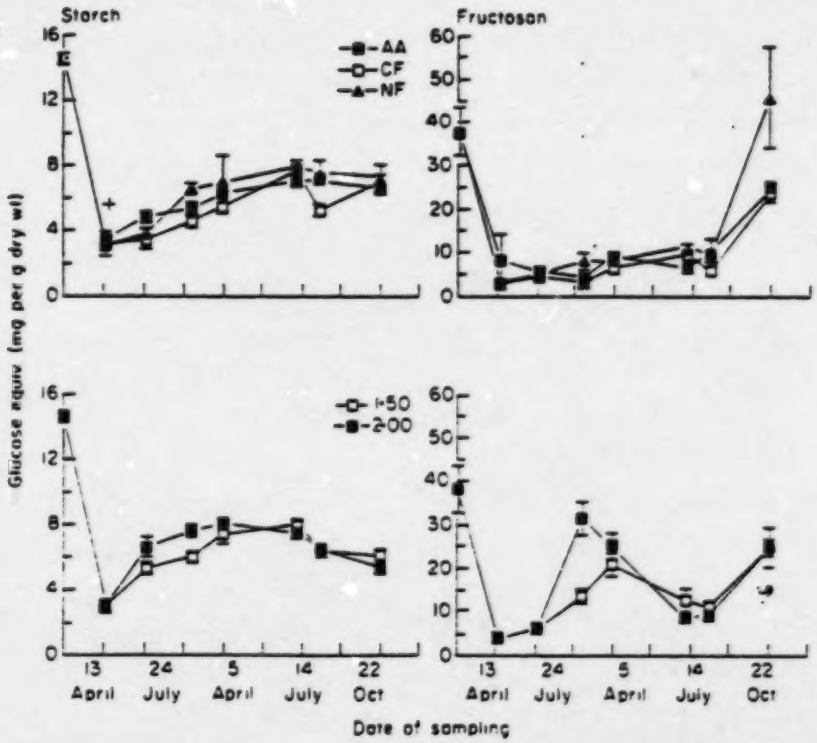

FIG. 7. Starch and fructosan (mg glucose equivalents g^{-1} dry weight) content in tall fescue shoots grown in either low (CF, AA, and NF) or high (1·50 × AA and 2·00 × AA) ozone air. Each point represents the mean of eight samples ± S.E. Statisucally significant treatment effects ($P < 0·05$) are indicated by the following symbols: * for O_3, + for moisture and × for O_3 × moisture effect. Values are combined over moisture levels. Abbreviations as in Fig. 2.

1·50 × AA), 15·5 (CF), and 21·4 (2·00 × AA) mg g^{-1} dry weight (L.S.D. at 5% level = 5·0). The only significant moisture level effect ($P < 0·05$) was observed for the final harvest, where WS clover grown in low O_3 air had 39% more starch (mg g^{-1} dry weight) than the WW treatments. No significant chamber effects were observed for starch content of clover shoots.

Clover shoots grown in high O_3 air had somewhat similar patterns in starch accumulation and depletion to clover grown in low O_3 air; however, the magnitudes of change were different (Fig. 6). A significant O_3 × moisture effect ($P < 0·005$) was only observed on 26 July 1984. In 1·50 × AA air, the starch content (mg g^{-1}) of clover shoots was 14·65 in WW plots and 20·92 in WS plots. In 2·00 × AA air, starch values were 19·49 in WW plots and 16·00 in WS plots (L.S.D. at 5% level = 3·69).

A steady accumulation of starch occurred in clover roots for plants grown in low O_3 during 1984 (Fig. 6). A depletion of starch reserves occurred over the winter reaching the lowest point (of all samplings over 2 years) on 5 April 1985. There was little build-up of starch reserves until autumn 1985 (22 October). This build-up occurred only in AA and

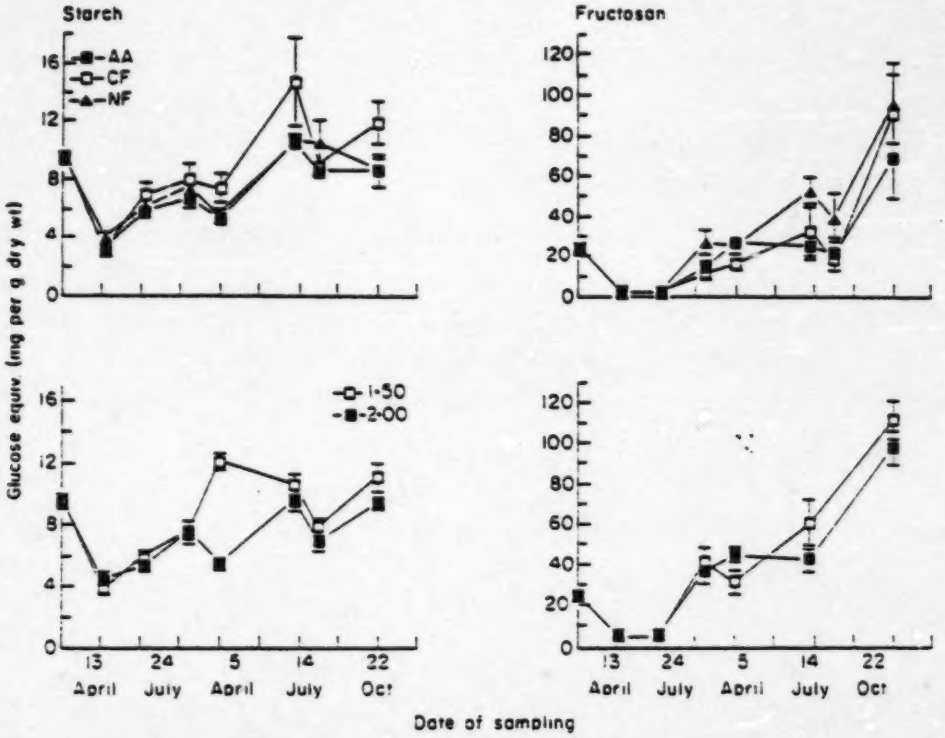

FIG. 8. Starch and fructosan (mg glucose equivalents g^{-1} dry weight) content in tall fescue roots grown in either low (CF, AA, and NF) or high (1·50 × AA and 2·00 × AA) ozone air. Each point represents the mean of eight cores ± S.E. Statistically significant treatment effects ($P < 0·05$) are indicated by the following symbols: * for O_3, + for moisture and × for O_3 × moisture effect. Values are combined over moisture levels. Abbreviations as in Fig. 2.

CF-treated plants, since no clover was recovered in samples from the NF plots. No significant chamber effects were observed. Significant O_3 ($P < 0·04$), moisture ($P < 0·011$) and O_3 × moisture ($P < 0·03$) effects were observed for the final 1985 harvest. Starch levels (mg g^{-1}) were highest in the CF + WS plots (186·2) when compared to the AA + WW (163·0) and CF + WW (157·1) plots. However, living clover roots were only recovered from AA + WS (only one starch determination) and CF plots on this date (L.S.D. at 5% level = 38·9).

Different patterns of starch accumulation and depletion were observed in the roots of clover grown in high O_3 air compared with low O_3 grown plants (Fig. 6). In the midsummer of 1984, a drop in starch content (26 July) occurred which was not observed in the low O_3 grown clover. A second larger starch peak was noted on 26 September 1984 in the high O_3 treatments. For 1985, starch content of roots steadily declined until the 22 October 1985 harvest where no live clover roots were found in the cores taken. No significant O_3, moisture or O_3 × moisture effects were observed.

Tall fescue. A large decline in starch content followed the initiation of O_3 treatments (including no chamber plots) in 1984 (Fig. 7). No clear seasonal changes in starch content

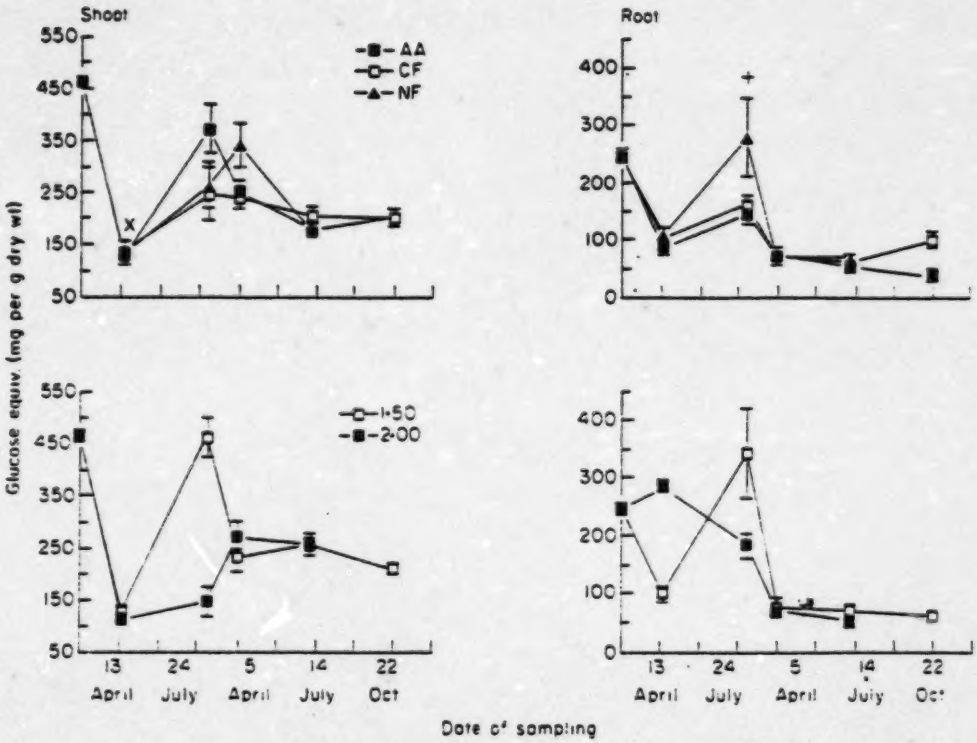

FIG. 9. Total soluble sugar (sum of glucose + fructose + sucrose) content (mg glucose equivalents g^{-1} dry weight) in ladino clover shoots and roots grown in either low (CF, AA, and NF) or high (1·50 × AA and 2·00 × AA) ozone air. Each point represents the mean of eight cores ± S.E. Statistically significant treatment effects ($P < 0·05$) are indicated by the following symbols: • for O_3, + for moisture and × for O_3 × moisture effect. Values are combined over moisture levels. Abbreviations as in Fig. 2.

were detectable in tall fescue shoots grown in either low or high O_3 air. Fructosan accumulation also declined rapidly for both low and high O_3 grown plants following the initiation of O_3 treatments in 1984 (Figs 7 and 8). In shoots of plants exposed to low O_3 levels, fructosan increased again in the fall of 1985 (22 October). Shoots of tall fescue grown in high O_3 air had a more cyclic nature to their fructosan reserves, i.e. fructosan peaked in autumn in both years (Fig. 6). No significant O_3 effects on starch and fructosan accumulation were observed. On 5 June 1984, significant moisture level effects on starch ($P < 0·05$) and fructosan ($P < 0·05$) levels were observed in water-stressed fescue shoots, which contained 28% higher starch (mg g^{-1} dry weight) and 49% higher fructosan levels than well-watered plants.

After the initial decline in 1984, starch levels of living fescue shoots grown in low and high O_3 increased slowly over the two growing seasons. No significant chamber, O_3, moisture and O_3 × moisture effects were observed. Fructosan levels in fescue roots also declined initially in 1984 and then gradually increased over the two growing seasons.

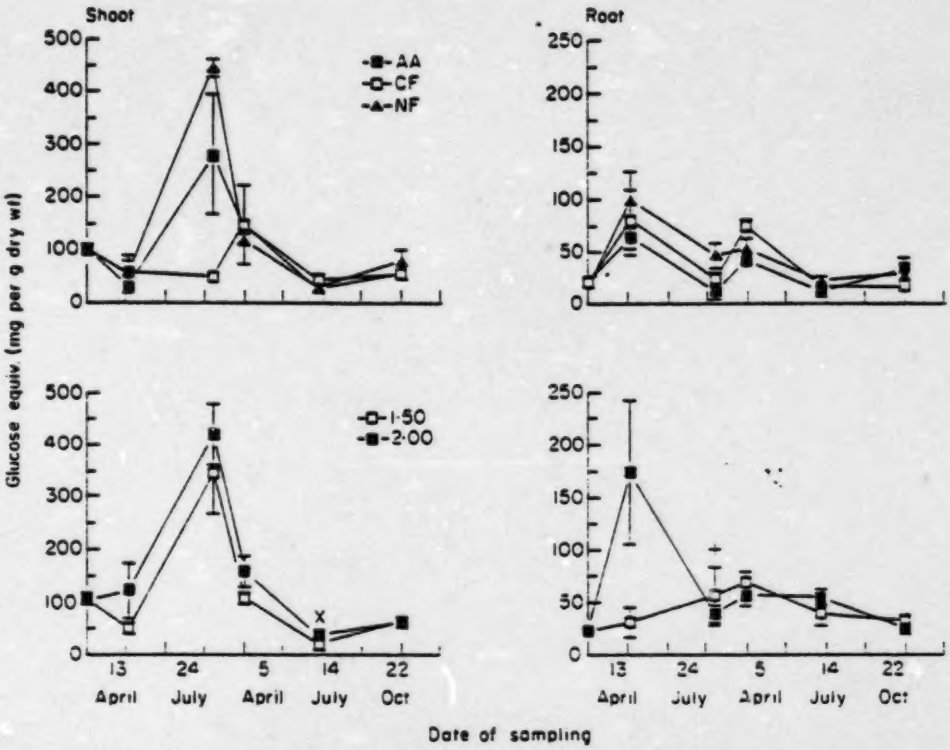

FIG. 10. Total soluble sugar (sum of glucose + fructose + sucrose) content (mg glucose equivalents g^{-1} dry weight) in tall fescue shoots and roots grown in either low (CF, AA, and NF) or high (1·50 × AA and 2·00 × AA) ozone air. Each point represents the mean of eight cores ± S.E. Statistically significant treatment effects ($P < 0·05$) are indicated by the following symbols: * for O_3, + for moisture and × for O_3 × moisture effect. Values are combined over moisture levels. Abbreviations as in Fig. 2.

Chamber ($P < 0·04$) and moisture ($P < 0·04$) effects were observed for fructosan reserves on 8 August 1985 in low O_3 grown plants. In low O_3 air, fructosan levels were 53% higher in WS fescue roots grown in low O_3 than in WW plants. In high O_3 grown fescue roots, a significant O_3 × moisture effect ($P < 0·05$) was observed for 8 August 1985 harvest. Fructosan (mg fructose g^{-1} dry weight) levels were highest in roots of fescue grown in 2·00 × AA + WS plots.

Total soluble sugars. Sucrose, glucose, and fructose in clover and fescue, and short-chain fructans in fescue were quantified only on the following dates: 13 April, 5 June, and 26 September 1984; and 5 April, 8 July, and 22 October 1985. Levels of total soluble sugars (sum of sucrose, glucose, fructose and, for fescue only, short-chain fructans are shown in Figs 9 and 10). Total soluble sugars (TSS) initially declined for clover in 1984 similar to the decline in starch. This however, was not the case for fescue where TSS were initially unchanged or increasing.

No significant O_3 or moisture effects were observed on TSS concentration (mg g^{-1} dry weight) in clover shoots. However, a significant $O_3 \times$ moisture effect ($P < 0.03$) was observed on 5 June 1984. In general, the WS clover shoots grown in low O_3 had 15% higher concentrations of TSS than the WW plants. No significant differences in TSS concentration due to O_3 or moisture levels were observed between 1·50 and 2·00 × AA-grown clover shoots or roots. In clover roots grown in low O_3 air, TSS concentrations were not significantly affected by O_3 ($P < 0.01$) until the final 1985 harvest. The living roots of CF-grown clover had a 62% higher TSS concentration than AA-grown roots (not enough clover was found in samples from NF plots to perform chemical analyses). Moisture had a significant effect ($P < 0.05$) only for the final 1984 harvest, where WS clover roots grown in low O_3 had a 48% higher TSS concentration than WW roots.

Levels of TSS in fescue shoots grown in low O_3 air were not affected by O_3 or moisture. On 8 July 1985, TSS levels of fescue shoots grown in high O_3 air were significantly affected by $O_3 \times$ moisture ($P < 0.02$) with 18·49, 27·83, 43·04 and 30·33 mg g^{-1} TSS for 1·50 × AA + WW, 1·50 × AA + WS, 2·00 × AA + WW and 2·00 × AA + WS, respectively. Peak concentrations of TSS in fescue roots, grown in both low and high O_3, were observed in June 1984 except for 1·50 × AA plants (peak on 5 April 1985). No significant O_3 or moisture effects on TSS concentrations of low O_3 grown fescue roots were observed. No statistically significant differences in TSS concentration were detected between 1·50 and 2·00 × AA-grown fescue roots. However, a significant moisture effect ($P < 0.0001$) was observed on 26 September 1984, with WS living fescue roots containing a higher TSS concentration (69%) than WW roots.

DISCUSSION

Estimates of the number of cores required to detect differences between two means of the root and shoot biomass of tall fescue and ladino clover indicate that there was a high amount of sample variance. Other researchers have reported high variability in root core samples taken in field studies (Troughton 1951; Williams & Baker 1957; Bohm 1979; Noordwijk, Floris & deJager 1985). Williams & Baker (1957) sampled grasslands of various species composition and reported the coefficient of variation of 10–320 samples (6–7 cm diameter cores) ranged from 19 to 36%. In this study, it would have been necessary to sample the entire chamber area excluding that area occupied by the permanent quadrats (*c.* 176 cores) on any date in 1984 to detect differences of 10 and 20% between biomass means of fescue and clover shoots and roots. To detect differences of 30 and 50% in the 1984 samplings, the necessary core number ranged from 6 to 106 or 2 to 118 in clover shoot and root, and 20–176 or 40–176 in fescue shoot and root, respectively. Similar core numbers were required in 1985 for clover. However, to detect differences of 30 and 50% between biomass means of fescue shoots and roots in 1985, the necessary core number ranged from 4 to 162 or 1 to 176, respectively. Ozone affected clover more than fescue, so clover was more common in the low O_3 plots. As a result, variability associated with clover biomass data was greatest in the high O_3 plots where the clover stand was irregularly distributed. Conversely, variability associated with fescue biomass means was lowest in the high O_3 plots.

It was difficult to make direct comparisons of the biomass production obtained from permanent quadrats and core harvests, since they represented such different portions of the population within the plots. Each individual permanent quadrat area equalled an area of 2532 cm^2 and a total of six were sampled from each plot at each harvest. Each

individual root core equalled an area of 102 cm^2 and two cores were randomly taken from each plot at each harvest. The core harvests would provide information more relevant to individual plants within a population while the permanent quadrats would provide information more relevant to the populations. Since the species composition varied across O_3 treatments and the areas sampled were different, the probability of finding clover, for example, in a sample from a high O_3 plot was substantially higher in the permanent quadrats than in root cores. Despite these differences, general changes in species composition and above-ground effects were consistent between the core and quadrat samplings. Chamber effects were observed only on fescue root and shoot biomass at a few samplings in 1985.

The seasonal patterns of shoot and root biomass of ladino clover and tall fescue observed (as represented by the NF-air-grown plants) are comparable to those observed in other field studies (Baker & Garwood 1959; Evans 1973; Davidson 1978; Wolf, Brown & Blaser 1979; Carlson, Gibson & Baltensperger 1985; Kendall & Stringer 1985). Both species exhibit their highest production in spring and autumn in the south-eastern U.S. For example, 50–60% of tall fescue biomass is produced before 1 June in North Carolina, U.S.A. (Mueller, Green & Chamblee 1984). Virtually no growth occurs in the summer months (Jacques & Schwass 1956; Baker 1957; Baker & Garwood 1959; Davidson 1978).

Soil moisture level had a significant effect on both species when the two soil water treatments were sufficiently different (i.e. during periods of low ambient rainfall), which occurred primarily in mid-1985. In most cases, living root biomass of both species was affected more than shoot biomass. However, statistically significant changes in R/S ratios were only observed in a few instances. Fescue living root biomass was significantly greater when water-stressed than when well watered. The reverse was true for clover. Taproots of ladino clover are short-lived (*c.* 1 year), and surviving stolons have relatively short fibrous roots which are incapable of deep soil penetration (Jones 1980; Westbrooks & Tesar 1955). Tall fescue has a longer, thinner, and more finely-branched root system than clover. Thus, under limiting soil moisture, white clover competes poorly with fescue for soil water. In addition, tall fescue is much more drought-tolerant than white clover (Hagan *et al.* 1957; Blake, Chamblee & Woodhouse 1966; Burch & Johns 1978; Turner & Begg 1978; Burch 1979; Haynes 1980; Johnson & Raguse 1985). Mild to moderate soil moisture deficits generally favour root growth over shoot growth (Davidson 1978).

Ambient and above-ambient levels of O_3 substantially modified the production and quality of the legume–grass pasture. Direct negative impacts of O_3 were clearly evident on clover shoot and root production. Indirect positive effects of O_3 were observed for fescue production. Because fescue is more tolerant than clover to O_3, the fescue population benefitted from the suppression of clover in high O_3 air. Numerous studies have demonstrated similar relative sensitivities of ladino clover and tall fescue (Kochhar, Blum & Reinert 1980; Flagler & Youngner 1982a & b; Blum, Smith & Fites 1982; Montes, Blum & Heagle 1982; Blum, Mrozek & Johnson 1983; Blum *et al.* 1983; Montes *et al.* 1983).

A number of reports indicate that root biomass is generally reduced more than shoots by O_3 (Blum, Mrozek & Johnson 1983; Blum, Smith & Fites 1982; Flagler & Youngner 1982a; Koziol 1984) which was not observed in this study until the second season. The greater impact on roots has been attributed to reduced energy fixation and a reduction in the proportion of total carbon translocated to roots. O_3 reduces net photosynthesis and increases or decreases respiration (Tomlinson & Rich 1967; Hill & Littlefield 1969; Tingey, Fites & Wickliff 1973; Blum, Smith & Fites 1982; Blum, Mrozek & Johnson 1983; Koziol 1984). Blum, Mrozek & Johnson (1983) also reported that the proportion of

carbon allocated to ladino clover roots increased gradually from 35 to 52% with increasing O_3 levels up to 0·10 $\mu l\ l^{-1}$ and then decreased to 21% at 0·15 $\mu l\ l^{-1}$. This may explain why shoots were affected before roots in 1984. It was not until 1985 that the impact of O_3 was greater on roots than on shoots of ladino clover grown in low O_3. The O_3 impact in 1985 on root growth was possibly a consequence of the declining status of starch reserves in the plant. Starch reserves already low due to O_3 treatments in 1984 were depleted further by overwintering, regrowth in the spring, and shifts of energy to stolon production. Starch reserves in clover roots for 1985 were never comparable to autumn 1984 levels. Only plants grown in CF air had carbohydrate reserves close to 1984 levels. Westbrooks & Tesar (1955) reported lower TNC levels in ladino clover roots in the second winter than during the first winter from seeding. They also observed increasing root mortality with time. However, they did not conclude that root mortality was a result of declining TNC, but that the lower TNC may have been the result rather than the cause of root mortality. In this study, the reduction in root growth observed in ambient and above-ambient O_3 could be largely responsible for the reduction in the energy reserves and storage capacity of the roots.

Total soluble sugar concentrations in 1985 did not vary in either shoots or roots. In general, O_3 had little effect on soluble sugar levels. A certain critical level of hexose sugars and sucrose may be maintained for cellular processes, and any excess or shortage may be expressed in changes in the storage carbohydrate levels (starch and fructosan). The maintenance of a critical TSS level has been reported in a number of grass species and some legumes (Brown & Blaser 1965; Smith 1972, 1973; White 1973; Pollock 1984). Presence or absence of seasonal patterns of soluble sugars, starch, fructosan, and TNC composition in legume shoot herbage appears to vary with the investigation (Smith 1972, 1973; White 1973). In this study, storage carbohydrate (starch in clover and starch + fructosan in fescue) reserves increased in autumn and decreased in the spring. This appears to be the typical pattern for most perennial grasses and legumes (Smith 1972, 1973; Leffel & Gibson 1973; White 1973; Carlson, Gibson & Baltensperger 1985; Kendall & Stringer 1985).

Tall fescue is a highly elastic species with the ability to survive over a wide range of soil and environmental conditions. Although the previously reported insensitivities to O_3 and drought were observed in this study, a few reports indicate that tall fescue can be affected by O_3 (Flagler & Youngner 1982a,b, 1985). For example, Flagler & Youngner (1982a) reported O_3 (0·30 $\mu l\ l^{-1}$ for 6 h day^{-1} once weekly for 12 weeks) significantly affected root–shoot ratios and dry weight. These O_3 concentrations are considerably greater than those found in south-eastern U.S.A. air or used in this study. Due to the dominance of clover in low O_3 plots in 1984, fescue shoot and root production was suppressed. However, when the clover populations were suppressed by O_3 and limited soil moisture, fescue populations were 'released'. This occurred in early 1984 in the high O_3 plots but was delayed until 1985 in the low O_3 plots. Fescue production was suppressed only in O_3 concentrations below ambient levels (CF air) for the entire study. Suppression could have been due in part to limited 'space' and availability of nitrogen in the low O_3 plots. Death and decay of clover was minimal in these plots and new space and nitrogen availability was greater in the high O_3 plots, since clover mortality was high and nitrogen released by decay of clover could be utilized by fescue.

Fescue shoot carbohydrates (storage and soluble sugars) were not significantly affected by O_3 exposures. This is consistent with earlier results (Flagler & Youngner 1985) in which greenhouse-grown tall fescue were exposed to 0·30 $\mu l\ l^{-1}$ O_3 for 6 h day^{-1} once a week for